Available online at www.sciencedirect.com

SCIENCE DIRECT

Virology 344 (2006) 378 – 390

VIROLOGY

www.elsevier.com/locate/yviro

Transcription and RNA processing during expression of genes preceding DNA ligase gene *30* in T4-related bacteriophages

Lidija Truncaite, Aurelija Zajančkauskaite, Aivaras Arlauskas, Rimas Nivinskas *

Department of Gene Engineering, Institute of Biochemistry, Mokslininku 12, 08662 Vilnius, Lithuania

Received 24 May 2005; returned to author for revision 1 July 2005; accepted 2 September 2005
Available online 12 October 2005

Abstract

Early gene expression in bacteriophage T4 is controlled primarily by the unique early promoters, while T4-encoded RegB endoribonuclease promotes degradation of many early messages contributing to the rapid shift of gene expression from the early to middle stages. The regulatory region for the genes clustered upstream of DNA ligase gene *30* of T4 was known to carry two strong early promoters and two putative RegB sites. Here, we present the comparative analysis of the regulatory events in this region of 16 T4-type bacteriophages. The regulatory elements for control of this gene cluster, such as rho-independent terminator, at least one early promoter, the sequence for stem-loop structure, and the RegB cleavage sites have been found to be conserved in the phages studied. Also, we present experimental evidence that the initial cleavage by RegB of phages TuIa and RB69 enables degradation of early phage mRNAs by the major *Escherichia coli* endoribonuclease, RNase E.

Keywords: T4-type bacteriophages; Early promoters; Genomic region *30.9–30.6*; RNA secondary structures; RNase RegB; RNase E

Introduction

T4-related bacteriophages constitute a type genus of the *Myoviridae* family. Based on the genomic hybridization data and PCR analysis, as well as on the comparisons of the sequences of three major virion structural proteins (gp18, gp19, and gp23), the T4-type phages can be further divided into four subgroups: the T-evens, the pseudo T-evens, the shizo T-evens, and the exo T-evens (Desplats and Krisch, 2003; Hambly et al., 2001; Tétart et al., 2001). Several of the T4-type phages (TuIa, RB69, and SV14) seem to occupy an "intermediate" position between T-even and pseudo T-even phages (Monod et al., 1997; Tétart et al., 2001; Yeh et al., 1998).

Bacteriophage T4 is the best-studied phage of the genus, but complete genome sequences of other representatives of different subgroups are now available (Miller et al., 2003a; http://phage.bioc.tulane.edu). Genomic comparisons have shown that T4-type genomes share homology for most of the essential genes, and their relative order is conserved. In general, there are homologues of T4 genes encoding structural proteins of the virion, the DNA replisome, and the late transcriptional apparatus. These data provide molecular evidence that T4-like phages are phylogenetically related. The major differences between phage genomes are in the regions of the genes whose function is either unknown or non-essential in T4. Some of them are absent, while others have been replaced by novel ORFs that are unique to the particular phage or phage subgroup. These genes are thought to be involved in the adaptation strategies of different phages (Desplats and Krisch, 2003; Miller et al., 2003a).

Comparisons of the genomes of T4-type phages have concentrated on the structural or other essential genes, while comparisons of the regulatory regions received relatively little attention. Nevertheless, these studies revealed significant plasticity in the intercistronic regions carrying regulatory elements (Loayza et al., 1991; Repoila et al., 1994; Yeh et al., 1998). Investigation of genomic sequences and sectional transcriptional analysis revealed that some T4-type phages had evolved different strategies for the control of their gene expression. It was found that the broad-host-range phages RB49 and KVP40 employ only two classes (early and late) of temporally regulated promoters (Desplats and Krisch, 2003; Miller et al., 2003a), rather than the three in T4 (Miller et al., 2003b). However, the mechanisms of transcriptional and post-

* Corresponding author. Fax: +370 5 2729196.
E-mail address: rimasn@bchi.lt (R. Nivinskas).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.09.001

transcriptional regulation of gene expression have not been explored in T4-type phages other than T4.

The infection cycle of T4 relies on the sequential expression of the clearly defined classes of the early, middle, and late genes that are transcribed by host RNA polymerase subsequently modified by phage proteins. T4 transcription is controlled primarily by three types of unique promoters that are activated in a time-dependent manner (Miller et al., 2003b). However, rapid shift of gene expression from early to middle and late stages also requires mRNA degradation or stabilization in a stage-dependent manner. Both host and phage-encoded factors cooperate in determining levels of T4 mRNAs. The only known T4-encoded endoribonuclease RegB takes place in the selective degradation and inhibition of translation of early T4 mRNAs (Ruckman et al., 1989; Sanson et al., 2000; Uzan et al., 1988). Many T4 mRNAs can be degraded or processed by primary *Escherichia coli* endoribonuclease, RNase E, which acts throughout phage development (Carpousis et al., 1989; Mudd et al., 1988, 1990; Otsuka et al., 2003). *E. coli* RNase III has also been implicated in the decay of a few specific phage mRNAs (Portier et al., 1987). Moreover, recent studies revealed a novel *E. coli* RNase LS (Otsuka and Yonesaki, 2005), which takes place in the T4 RNA metabolism under the control of T4 gene *dmd* product, which functions to discriminate mRNAs for degradation in the stage-dependent manner (Kai et al., 1996, 1998; Kai and Yonesaki, 2002; Otsuka et al., 2003; Ueno and Yonesaki, 2001). It is also indicative that some T4-coded proteins alter the specificities of RNases E and G towards the phage and host RNAs after infection (Ueno and Yonesaki, 2004).

To compare the regulatory events during early stages of infection of different T4-type bacteriophages, we used structure–function analysis of the regulatory region for the genes clustered in the direction of early transcription together with the essential gene *30*. This T4 gene codes for DNA ligase, which is involved in the T4 DNA metabolism. The orthologues of this gene, as well as the other genes of the T4 replisome, could be found in almost all T4-type phages sequenced to date. However, the regulation of this gene expression is not well established even in T4.

Previously, we investigated the transcriptional regulation of gene *30* and found that it is first transcribed from middle promoter P_M30, but transcription of this essential gene is also under control of other middle promoter, $P_M30.2$, and under control of more distant MotA-independent promoters as well (Truncaite et al., 2002). We speculated that the gene *30* could be transcribed into the long polycistronic transcripts directed from early promoters $P_E30.8(128.6)$ and $P_E30.7(128.2)$. These strong early promoters located downstream from the rho-independent terminator (t128.6) after the gene *30.9* have been mapped on T4 genome by in vitro runoff transcription (Gram et al., 1984), by probing transcription from cloned fragments (Liebig and Rüger, 1989) and by sequencing (Nivinskas et al., 1992). However, the activities of these promoters during phage development have never been demonstrated. Furthermore, from the sequence data, we detected two putative sites of RegB endoribonuclease in the Shine–Dalgarno (SD) regions of genes *30.8* and *30.7*. Primer extension analysis of the transcription in this region could reveal the regulatory events, which really take place during T4 infection.

Therefore, the aim of this study was to examine the activities of T4 early promoters $P_E30.8(128.6)$ and $P_E30.7(128.2)$ during T4 infection and to test whether the RegB is involved in the post-transcriptional regulation of the genes from this transcription unit. Furthermore, we wished to realize whether the regulatory elements, as well as the regulatory pattern revealed during T4 infection, are common to the phages of the T-even family. Therefore, we determined the nucleotide sequences of this regulatory region in 16 T4-related bacteriophages and studied the organization, transcription, and RNA processing from this region of the phages.

Results

Transcriptional and post-transcriptional control of the expression of T4 genes 30.8 and 30.7

Based on microarray analysis of T4 gene transcription, most of the genes clustered in the direction of transcription towards the DNA ligase gene *30* have been assigned to the early temporal class (Luke et al., 2002). This was consistent with the earlier in vitro studies that had mapped classical rho-independent transcription terminator t128.6 and two early promoters, $P_E30.8(128.6)$ and $P_E30.7(128.2)$, in the regulatory region for this gene cluster (Gram et al., 1984; Nivinskas et al., 1992). Both promoters share features of strong T4 early promoters that are defined by the highly conserved −35 region GTTTAC(a/ttt), the conserved and extended −10 region TGnTA(t/c)(a/t)AT, and the spacing of 16 to 17 bp between them (Liebig and Rüger, 1989; Wilkens and Rüger, 1994, 1996). The T4 genome contains 39 early promoters with the characteristic sequence (Miller et al., 2003b; Wilkens and Rüger, 1994; Sommer et al., 2000). Most of them have been identified by in vitro runoff transcription (Gram et al., 1984) or using plasmid vectors (Liebig and Rüger, 1989). To examine the activities of early promoters $P_E30.8(128.6)$ and $P_E30.7(128.2)$ in vivo, we analyzed the mRNAs of T4 genes *30.8* and *30.7* isolated from *E. coli* cells after infection with phage $T4D^+$.

Fig. 1 shows the results of primer extension sequencing and the analysis of the 5′-end accumulation kinetics of gene *30.8* mRNAs in the T4-infected cells. Primer extension analysis of T4 gene *30.8* transcripts revealed several species of bands reflecting the reverse transcriptase (RT) stops. A couple of bands at the top of the autoradiograph should be assigned to the stops at the stem-loop structure of the terminator t128.6 carried by the read-through transcripts. Transcription induced from early promoter $P_E30.8(128.6)$ starts at A positioned 6 bp downstream from −10 region. As for typical early gene, transcription of gene *30.8* starts immediately after infection, but the amount of the transcripts diminishes at 2 min after infection (Fig. 1A). The truncated transcripts appear at the same time, and they begin to

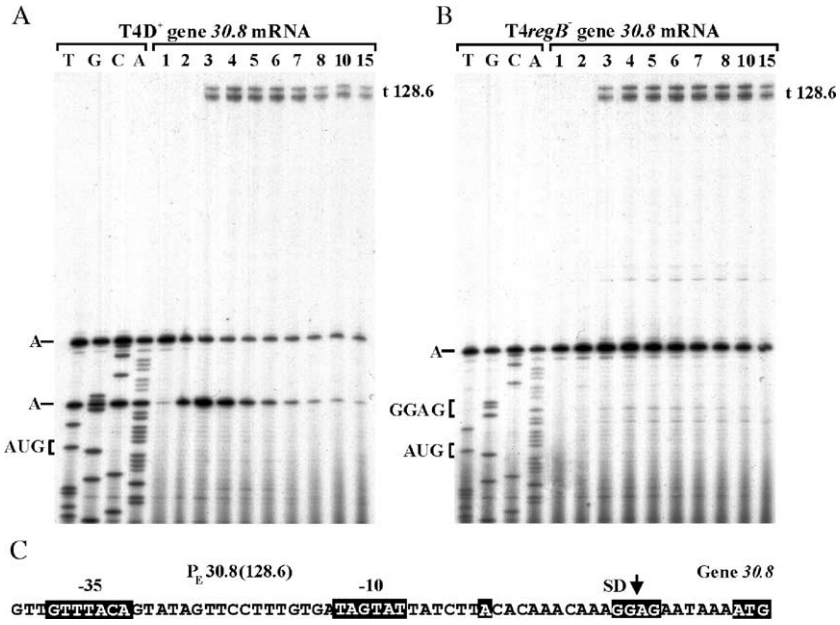

Fig. 1. Primer extension analysis of gene *30.8* mRNA using RNA isolated from the *E. coli* B^E cells infected with phage T4D wild type (A) or its mutant T4*regBL52* (B) at 30 °C. Primer extension sequencing of RNA isolated at 3 min post-infection and the kinetics of the 5′-end accumulation of RNA isolated at 1 to 15 min post-infection. The sequencing lanes are labeled with the dideoxynucleotides used in the sequencing reaction. The time (min) of post-infection that each RNA was isolated is noted at the top of the Figure. The 5′-end nucleotides of the transcripts, the GGAG motif within the SD sequence, as well as the initiation codon for the gene, are noted. (C) Nucleotide sequence of the 5′ flanking region of gene *30.8* of phage T4. The −35 and −10 elements of the early promoter, the start nucleotide for the transcript, the GGAG motif of the SD sequence, as well as the initiation codon for the gene, are shown in black background. The arrow denotes the position of RegB cleavage.

diminish from the fourth min after infection. The truncation occurs in the middle of the SD sequence motif GGAG suggesting that the gene *30.8* transcripts are subjected to the cleavage by the T4-induced RegB endoribonuclease. To confirm that RegB cleaves within the SD sequence of the transcript for gene *30.8*, we analyzed the transcription of this gene in the T4 *regB*$^-$ mutant, T4*regBL52*, infected cells. In this case, only the RT stop corresponding to the transcription start appears in the primer extension analysis (Fig. 1B). The transcripts for gene *30.8* in T4*regBL52* infection are much more stable, and slight decrease in their amount is observed only from seventh min post-infection.

Primer extension analysis of the transcripts for gene *30.7* was used to determine the in vivo activity of promoter P_E30.7(128.2). Fig. 2A shows the reactions of sequencing and accumulation kinetics of gene *30.7* mRNA in the T4-infected cells. The sequencing bands running upstream of the initiating nucleotide indicate that gene *30.7* is transcribed from both, P_E30.8(128.6) and P_E30.7(128.2), early promoters. Transcription initiated from the P_E30.7(128.2) starts with G, 6 bp downstream from −10 element, and shows the similar pattern, as that observed for gene *30.8*: the transcripts appear immediately after infection and reach their maximal amount at the first min after infection. The additional RT stop corresponding to the gene *30.7* mRNA truncated at the middle of SD motif GGAG appears at 2 min post-infection. Primer extension analysis of gene *30.7* mRNA synthesis in T4*regBL52*-infected cells has also confirmed the activity of the T4-induced RegB endoribonuclease towards the transcripts of gene *30.7* (Fig. 2B).

The sequence and organization of the regulatory region for the transcription unit starting with the genes 30.8 and 30.7 in T4-related bacteriophages

PCR analysis using the oligonucleotide primers based on phage T4 or RB69 genome sequences lets us suppose that most of the genes clustered upstream DNA ligase gene *30* were present in the genomes of the 16 T4-type phages tested. To obtain information about conservation versus divergence of the regulatory signals for these genes, we sequenced the segment of this gene cluster, which was supposed to contain the regulatory elements for control of gene expression, in these phages. Fig. 3 shows the schematic outline based on the sequence data obtained. The high level of sequence conservation in this region is observed in the genomes of 13 phages studied. The sequences for the rho-independent terminator after the gene *30.9* are near-identical to the corresponding sequences of T4 (Fig. 4A). When translated, gene *30.8* and *30.7* products of these phages share homology of more than 95% with their respective T4 homologues. The -10 and -35 elements of the T4 P_E30.8(128.6) analogues are identical to the conserved elements of their T4 counterpart, and only the spacing region between them contains few base differences or an insertion in case of phage M1 (Fig. 5A). The sequences of the T4 promoter P_E30.7(128.2) analogues in all phages are almost identical to their T4 counterpart (Fig. 5C). Also, despite numerous nucleotide differences, insertions or deletions in the non-coding region downstream of gene *30.7*, there are palindromic sequences giving rise to the RNA stem-loop in all phages (Fig. 4B). Moreover, in most cases, this RNA secondary structure

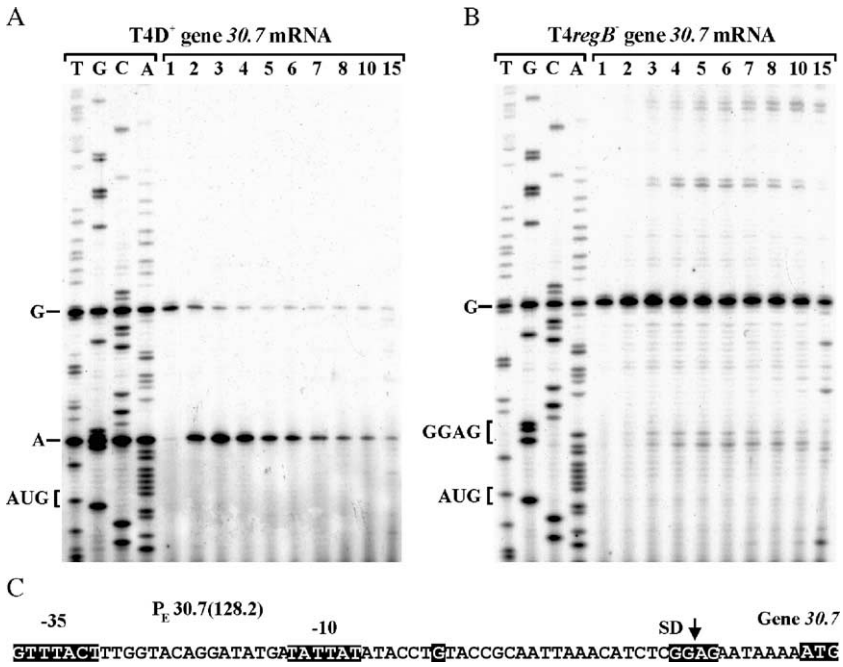

Fig. 2. Primer extension analysis of gene *30.7* mRNA using RNA isolated from the *E. coli* B^E cells infected with phage T4D wild type (A) or its mutant T4*regBL52* (B) at 30 °C. (C) The nucleotide sequence of the 5 flanking region of gene *30.7* of phage T4. Conditions and labels are as for Fig. 1.

has the predominant tetraloop sequence UUCG that confers the exceptional stability to the RNA hairpins (Tuerk et al., 1988).

Notable differences in the sequence and organization of this genomic region have been detected for the phages RB69, TuIa, and LZ10 (Fig. 3). The genomes of phages RB69 and TuIa encode an RNA hairpin structure with different nucleotide sequence from the hairpin that has been implicated in transcription termination in T4 (Fig. 4A). The phage RB69 has two early promoters in this region. The sequence of $P_E30.8_{RB69}$ has different promoter elements from $P_E30.8(128.6)$, and the spacing region between −35 and −10 elements consists of 16 bp. Therefore, this promoter is more similar to $P_E30.7(128.2)$ of T4 (Figs. 5B and C). Gene *30.8* of this phage has multiple differences from the T4 gene *30.8*, and it is longer than its T4 counterpart by 39 bp. The deduced primary structure of the RB69 gp30.8 has only 39.9% sequence identity with the T4 gp30.8. The −35 and −10 elements of $P_E30.7_{RB69}$ are identical to $P_E30.7(128.2)$ of T4 (Fig. 5C). The gene *30.7*, in comparison to T4, contains more differences than the corresponding gene of the other phages, but the deduced

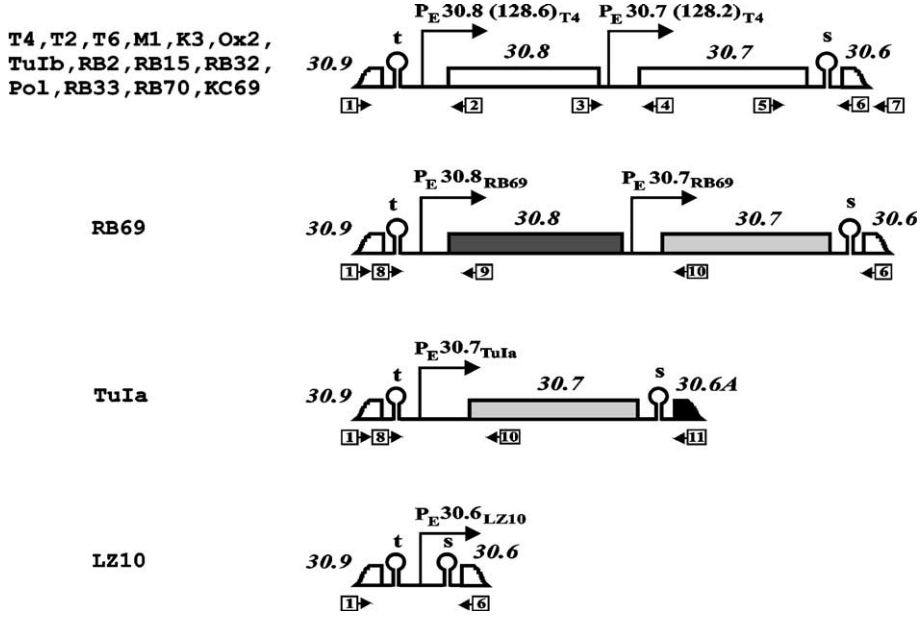

Fig. 3. Genes and intergenic elements in the region between *30.6* and *30.9* of 17 T4-related bacteriophages. Shown are the positions of genes, as well as the positions of rho-independent terminators (t), early promoters (P_E) and the putative stem-loop structures (s). The boxed numbers with arrows show the positions of primers used in the sequencing or primer extension reactions.

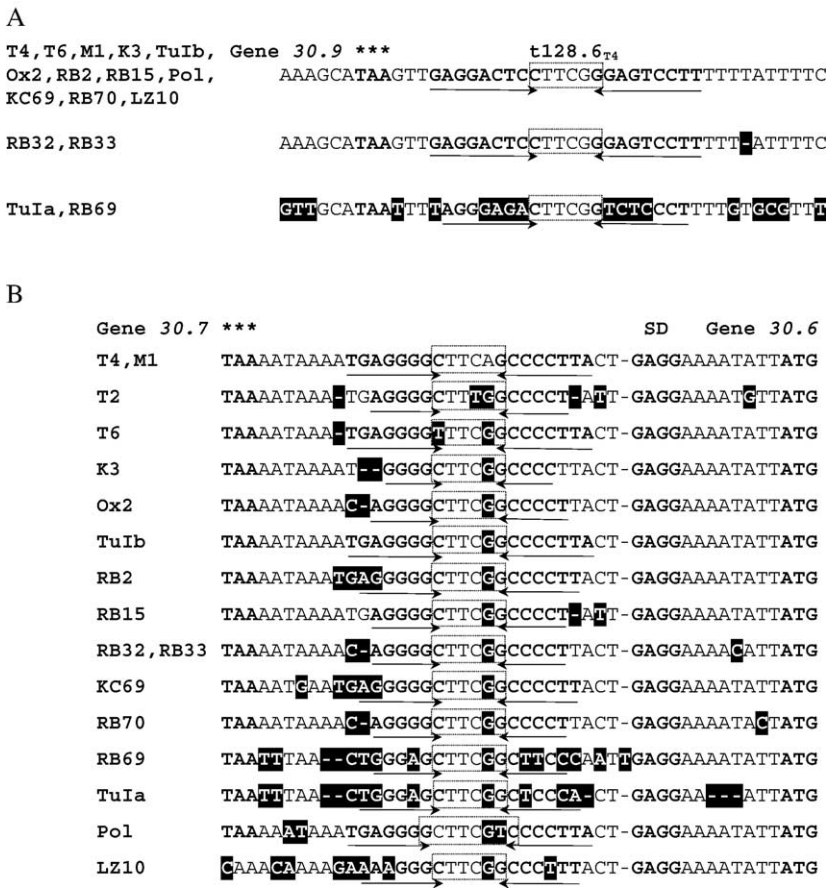

Fig. 4. Comparison of the nucleotide sequences of transcription terminator after gene *30.9* (A), and the non-coding region between genes *30.7* and *30.6* (B) of 17 T4-related bacteriophages. Different nucleotides are given in black background. A dash indicates a space, which was inserted in the sequence to preserve the alignment. Asterisks denote the termination codons of genes *30.9* and *30.7*. The initiation and termination codons, the GGAG motifs of the SD sequences, and the inverted repeats of palindromic sequences are given in bold. The inverted repeats of palindromic sequences are underlined by convergent arrows, the loop motifs are boxed.

primary structure of gp30.7 of RB69 shares about 94% sequence identity with its T4 counterpart.

Phage TuIa has only one early promoter in this region ($P_E30.7_{TuIa}$), which is like $P_E30.8_{RB69}$ (Figs. 3 and 5B). The second early promoter, as well as the gene *30.8*, are absent from this genomic region of TuIa. Gene *30.7* is almost identical to the gene *30.7* of RB69. Untranslated region after gene *30.7* in phages TuIa and RB69 exhibits a high degree of divergence, but there remains the palindromic sequence to form a stable RNA hairpin structure (Figs. 3 and 4B). The palindromic sequences for a stable stem-loop are in this region of phage LZ10 as well. The latter phage has the same sequence for the rho-independent terminator, as in T4, but contains only one early promoter, which is very similar to the $P_E30.8(128.6)$ of T4. Interestingly, there are no gene homologues of genes *30.8* and *30.7* in this region, and the promoter appears to be situated between the sequences encoding two stable RNA hairpins (Figs. 3 and 4).

5′-end mapping of the transcripts for genes 30.8, 30.7, and 30.6 of T4-related bacteriophages

As some T4-type phages of 16 tested showed differences in the promoter sequences in the region of interest, we examined their function during infection of these phages. First, we tested the activity of promoter $P_E30.6_{LZ10}$, which was situated between two pairs of palindromic repeats giving rise to two stable hairpins flanking the promoter sequence. Primer extension analysis of LZ10 gene *30.6* transcripts showed that this promoter was functional during infection of LZ10, and the kinetics of accumulation of the transcripts for gene *30.6* of LZ10 was similar to that usually found for early T4 genes (data not shown).

Two interesting variants of promoters have been found upstream of the gene *30.8* in the genomes of phages M1 and RB69 (Figs. 5A and B). The promoter $P_E30.8_{M1}$ had unusually long spacer region (18 bp) between conserved elements. Early promoter carrying typical −35 and −10 elements with the spacing of 18 bp has never been found in T4. The promoter $P_E30.8_{RB69}$ had a spacing of 16 bp and was more similar to $P_E30.7(128.2)$ of T4. Fig. 6 shows primer extension sequencing of the gene *30.8* mRNAs of phages T4, M1, and RB69. These reactions demonstrate that, in spite of the differences, the promoters $P_E30.8_{M1}$ and $P_E30.8_{RB69}$ are functional during early period of infection of these phages, but $P_E30.8_{M1}$ is weaker (Fig. 6). The mRNAs for gene *30.8* of these phages are also processed in the middle of the GGAG motif of their SD sequences.

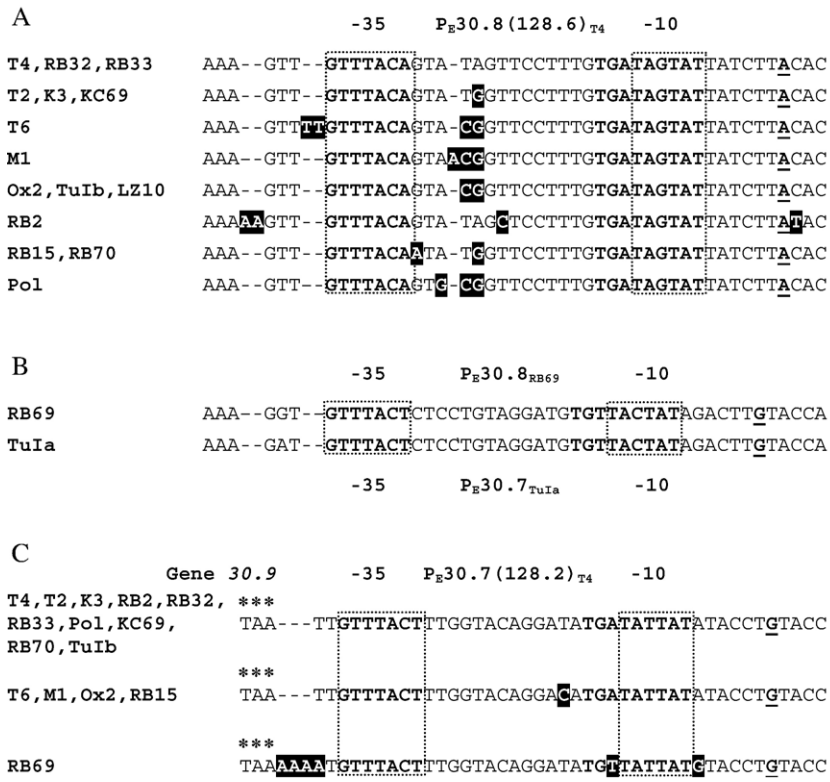

Fig. 5. Comparison of the nucleotide sequences of early promoters $P_E(30.8)$ and $P_E(30.7)$ of T4-related bacteriophages. (A) The sequences of $P_E(30.8)$ of T-even phages. (B) The sequences of promoters $P_E(30.8)_{RB69}$ and $P_E(30.7)_{TuIa}$. (C) The sequences of $P_E(30.7)$ of T4-related bacteriophages. Different nucleotides are given in black background. A dash indicates a space, which was inserted in the sequence to preserve the alignment. The −35 and −10 consensus elements of early promoters are boxed. The sequences of −35 and the extended −10 elements are shown in bold. The initiating nucleotide is shown in bold and underlined.

The genome of bacteriophage TuIa appeared to have only one early promoter in this region (Fig. 3). The sequence of this promoter of TuIa is identical to the $P_E30.8$ of RB69 (Fig. 5B). Since this promoter is situated upstream of the gene *30.7* homologue, we performed primer extension analysis of the transcripts for this gene. Fig. 7A shows the results of primer extension sequencing and the analysis of transcription kinetics of gene *30.7* of TuIa. The RT stop corresponding to the transcription initiation from $P_E30.7_{TuIa}$ is strongest at 1 min after infection and decreases thereafter. Transcription starts with G, 6 nt downstream from the −10 motif of promoter.

The transcripts of the gene *30.7* of TuIa appear also to be processed in the middle of the SD motif GGAG. The 5′-truncated transcripts weakly appear at 1 min after infection, become most abundant at 3 to 4 min, and start to decrease in their yield at 5 min after infection. Moreover, primer extension reactions performed on TuIa gene *30.7* mRNA revealed additional RT stop (A(2) in Fig. 7), which is weakly detected at 3 min after infection reflecting the cut within the RegB-processed transcripts. Cleavage takes place in A-rich sequence 3 nucleotides downstream from the cleaved GGAG site. The 5′-ends assigned to the additional cleavage event reach their maximum amount at 6 min post-infection and continue to accumulate onward. We also performed primer extension analysis of the transcripts for gene *30.7* of bacteriophage RB69. The analysis revealed the analogous sites for the transcript initiation and processing, as in case of TuIa gene *30.7* (data not shown).

Our previous experiments showed that phages M1, TuIa, and RB69 encode the homologues of endoribonuclease RegB that retain their specificities to the GGAG motifs (Piešiniene et al., 2004). Therefore, we conclude that the transcripts for genes *30.7* and *30.8* of these phages are cleaved by phage-coded RegB endoribonuclease. The question regarding the secondary cleavage event concerns which endoribonuclease, RegB itself or *E. coli* encoded nuclease is responsible for this activity. In the previous study mentioned above, we detected the activity of the *E. coli* RNase E towards early transcripts of the pseudo T-even phage RB49. Here, we next studied the possibility of the involvement of RNase E in the degradation of the RegB-cleaved early transcripts of the intermediate-type phages TuIa and RB69.

Investigation of the nature of the secondary cuts within RegB-processed gene 30.7 transcripts of bacteriophages TuIa and RB69

To investigate the origin of secondary cuts within the RegB-processed transcripts for gene *30.7* of bacteriophage TuIa, we analyzed the mRNA for this gene isolated 6 min after infection with TuIa in the presence or in the absence of chloramphenicol. Chloramphenicol was added 1 min before infection for inhibition of the phage protein synthesis. Primer

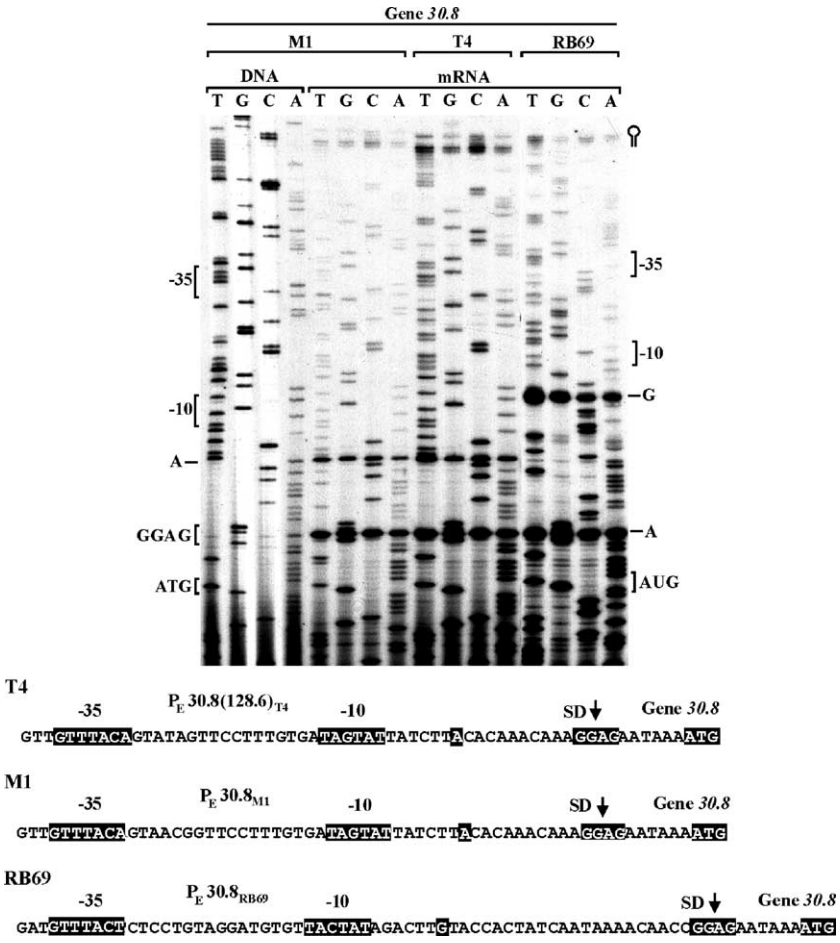

Fig. 6. Primer extension sequencing of the mRNAs for gene *30.8* of phages T4, M1, and RB69. Primer extension sequencing reactions were done on RNAs isolated at 5 min post-infection from *E. coli* B^E cells that were infected with phages T4, M1, or RB69 at 30 °C. Sequencing of DNA fragment carrying gene *30.8* of phage M1 is also presented. The sequencing lanes are labeled with dideoxynucleotides used in the sequencing reactions. The consensus elements of early promoters, the initiating nucleotide for the transcript, the GGAG motif within SD sequence, the initiation codon for gene *30.8*, and the 5′-end nucleotide for RegB-truncated transcript are noted. The nucleotide sequences of the 5′ flanking region of gene *30.8* of phages T4, M1, and RB69 are shown in the bottom of the Figure. The −35 and −10 regions of the early promoters, the 5′-end nucleotides for the transcripts, the GGAG motifs within the SD sequences, as well as initiation codons, are shown in black background. Vertical arrows denote the position of RegB cleavage.

extension sequencing reactions of such mRNAs are shown in Fig. 7B. The bands labeled (1)A and (2)A correspond to the RegB primary cleavage at the SD motif GGAG and the secondary cut within RegB-processed transcripts for gene *30.7*, respectively. In the reaction on mRNA isolated from the chloramphenicol-treated cells after infection with TuIa, only the transcriptional start is detected as a 5′-end (Fig. 7B). These experiments demonstrate that in the absence of phage protein synthesis, the transcript for gene *30.7* of TuIa becomes resistant to the nucleolytic attack at both the primary and the secondary sites.

To test whether the mRNA of TuIa gene *30.7* truncated at the secondary site could be the product of *E. coli* RNase E cleavage, total RNA was extracted from isogenic *E. coli* wild type and *rne* (ts) strains after infection with phage TuIa. The extracted mRNAs were analyzed by primer extension sequencing. In the wild-type background, the truncated mRNA of TuIa gene *30.7* was detected at either 30 °C or 43 °C (Fig. 7C), while in the *rne* (ts) background at 43 °C, only the 5′-end truncated at the GGAG was observed. The absence of the secondary cut in *rne* (ts) mutant at 43 °C indicates that the host-encoded RNase E generates the 5′-end at this site. We also analyzed RNA isolated from the *E. coli rne* (ts) strain after infection with RB69 at 30 °C and at 43 °C. The 5′-end assigned to the secondary cut has been observed for RB69 gene *30.7* transcripts isolated at 30 °C but disappeared in case of the transcripts isolated at 43 °C (data not shown). This experiment confirmed that the *E. coli* RNase E was responsible for the cutting within A-rich sequence located downstream from the RegB-processed primary site of TuIa and RB69 gene *30.7* mRNAs.

Discussion

The regulatory elements for the early gene cluster upstream DNA ligase gene 30 in T4-related bacteriophages

In this study, we examined the regulatory region that directs the expression of the early genes clustered upstream DNA ligase gene *30* in 16 T4-related phages. Structure–function analysis revealed that all phages employ similar regulatory elements for the genes carried by this transcription unit. All

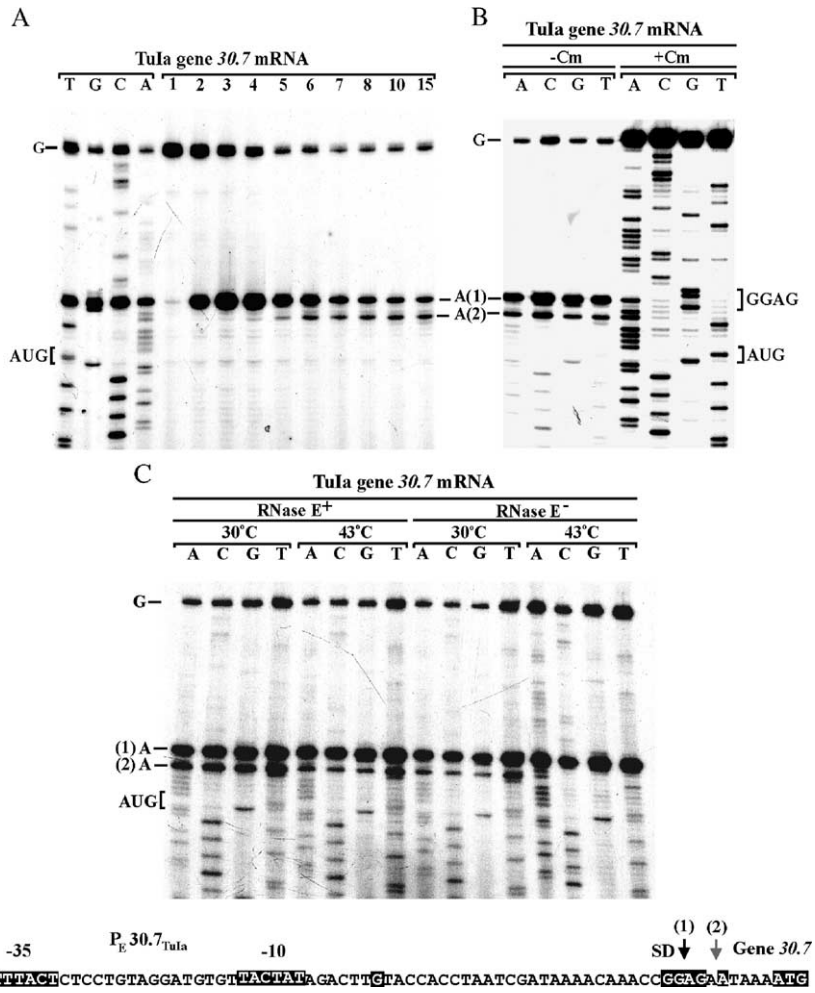

Fig. 7. Primer extension analysis of gene *30.7* mRNA of phage TuIa. (A) Primer extension sequencing of gene *30.7* mRNA isolated from the *E. coli* B^E cells infected with phage TuIa at 2 min post-infection at 30 °C, and kinetics of the 5′-end accumulation of gene *30.7* mRNA isolated at 1 to 15 min post-infection. The time (min) of post-infection that each RNA was isolated is noted at the top of the figure. (B) Primer extension sequencing reactions of gene *30.7* mRNA isolated from *E. coli* B^E cells at 6 min post-infection with TuIa in the absence or in the presence of chloramphenicol at 30 °C. (C) Primer extension sequencing of gene *30.7* mRNA isolated from *E. coli* N3433 (rne^+) and N3431 (rne^-) cells at 10 min post-infection with TuIa at 30 °C and 43 °C. The sequencing lanes are labeled with dideoxynucleotides used in the sequencing reactions. The transcriptional start nucleotide, the 5′-ends generated by RegB-dependent primary (1) and secondary (2) cleavages, the GGAG motif of SD sequence, as well as the initiation codon, are indicated. The nucleotide sequence of the 5′ flanking region of gene *30.7* of phage TuIa is shown at the bottom of the figure. The −35 and −10 regions of the early promoter, the start nucleotide for the transcript, the GGAG motif within SD sequence, the 5′-end nucleotide for RegB-processed transcript (1) and for the RNase E-processed transcript (2), as well as the initiation codon for gene *30.7*, are shown in black background. Arrows denote the positions of primary (1) and secondary (2) cleavages.

phages contain the sequences for the rho-independent terminator, at least one early promoter and the sequences for the RNA stem-loop structure upstream of gene *30.6*. The early promoters of 13 phages tested are very similar to their respective T4 counterparts and, except for $P_E30.8_{M1}$, match consensus sequence of strong T4 early promoters. It should be noted that most of the phages (14) studied belong to the T-even subgroup. Therefore, it is indicative that early promoters within this subgroup share similar features with the early T4 promoters. On the other hand, the promoters of phages RB69 and TuIa, which belong to the intermediate subgroup between T-evens and pseudo T-evens, have differences from their T4 counterparts but also share features of typical early T4 promoters. Interestingly, the promoters of these phages, $P_E30.8_{RB69}$, $P_E30.7_{RB69}$, and $P_E30.7_{TuIa}$, have 16 bp spacers between the consensus elements and are very similar (Fig. 5), indicating that they may have evolved by duplication of the same promoter.

The determined primary structures of the coding sequences from this region of T4-like phages provide the first orthologues of genes *30.8* and *30.7*. Neither of these genes has been identified in other T4-like genomes sequenced. The data show that the genes *30.8* and *30.7* of RB69 encode proteins of similar size (123 and 121 aa, respectively) with the first few amino acids being almost identical. This raised up the idea that the two genes had also arrived by duplication. However, the primary structures of gp30.8 and gp30.7 of both T4 and RB69 phages share identity of less than 30% (when calculated using ClustalW or Allign). To get better insight about the similarities of these proteins, we used the program T-Coffee, which is more accurate for the sequences with less than 30% identity (Notredame et al., 2000). The score showed good to average

similarity for the T4 gp30.8 and gp30.7, while the homologous proteins of RB69 showed good similarity to each other only in the region of six N-terminal amino acids. Therefore, the genes may have duplicated in T4 and diverged significantly during evolution.

The possibility of duplication is strengthened by the finding that both of the genes, *30.8* and *30.7*, are regulated by similar transcription–translation relations. The transcripts for the genes *30.8* and *30.7* appeared to belong to a subclass of prereplicative mRNAs that are subject to cleavage within GGAG motifs of their ribosome binding sites (RBS) by a phage-induced endoribonuclease RegB. The RegB involvement in the post-transcriptional regulation of these genes was unknown previously even in T4. Thus, we mapped two additional RegB-processing sites on the T4 genome, which was known to contain 22 RegB sites to date (Sanson and Uzan, 1995). The RegB target sequence GGAG is found frequently in translation initiation sites of early transcripts (Sanson and Uzan, 1995). By destroying RBS sites, RegB leads to functional inactivation of mRNAs. In support, our results show that the amounts of intact mRNAs for genes *30.8* and *30.7* start to decrease at 2 min after infection, while in the T4*regB*$^-$ infection their longevity is much greater. It should be noted that RegB sites in the SD regions of gene *30.8* and *30.7* homologues are absolutely conserved between phages tested suggesting that the products of these genes fulfill similar functions during early period of infection of T4-type phages.

In addition to the transcription initiation and processing events, some read-through transcription was observed in the reverse transcription experiments of T4 gene *30.8* mRNAs indicating an apparent lack of efficient transcription termination at terminator t128.6 (Fig. 1). The RT stops at the secondary structure of the terminator have been observed in the reverse transcription experiments with gene *30.8* mRNAs of phages RB69, M1, and TuIa (Fig. 6). Therefore, the analysis of the transcription pattern of this region indicates that the genes comprising transcriptional units could be expressed from either of two promoters or served by read-through across a termination site producing overlapping transcripts.

Transcription from T4 early promoters P_E30.7(128.2) and P_E30.8(128.6) produces a transcript with a highly stable RNA hairpin in the intergenic region between genes *30.7* and *30.6*. This untranslated region exhibits a high degree of divergence in the genomes of other phages, but different nucleotide sequences encode stable RNA hairpins in every case. Interestingly, most of the phages tested have CUUCGG-loop sequence, which is known to dramatically stabilize the hairpins (Tuerk et al., 1988). Such stable CUUCGG-carrying hairpins have been found to be ubiquitous in the genome of *E. coli* or even of eucaryotes. It was proposed that the similarity over such a short region may be due to convergent evolution rather than common descent (Bernstein and Bernstein, 1989), but specific function to such RNA hairpin structures has not been assigned.

Maintenance of the palindromic sequences in the intergenic region between genes *30.7* and *30.6* in T4 relatives indicates that this RNA structure may be important for the expression of the genes in this cluster. In many prokaryotes, the sequences capable of folding into stem-loop structures located in the 3′ non-coding regions have been implicated in protecting of the upstream mRNAs from the non-specific degradation by exonucleases (Kushner, 2002; Deutscher and Li, 2001; Zuo and Deutscher, 2001). However, here, we show that all phages employ the mechanism of RegB-mediated acceleration of degradation for the transcripts of their gene *30.7*. On the other hand, our previous experiments indicated that long polycistronic transcripts for genes *30* and *30.2* directed from MotA-independent promoters remain stable over a period of approximately 10 min of infection (Truncaite et al., 2002). Therefore, it is tempting to propose that this stem-loop structure might be involved in the stabilization of mRNA for the genes located downstream gene *30.6*. The RegB activity, which promotes specific degradation of genes *30.8* and *30.7*, and this mRNA stem-loop might contribute to the differential expression of the genes encoded by this polycistronic mRNA. Deletion of the gene *30.8* and *30.7* counterparts together with one promoter in this region of phage LZ10 genome does not eliminate the palindromic repeats supporting the idea that this RNA secondary structure evolves together with the downstream genes of this transcription unit.

Cooperative action of phage-coded RNase RegB and E. coli RNase E in the degradation of early transcripts of T4-related bacteriophages

The RegB-processed transcripts for gene *30.7* of phages TuIa and RB69 undergo further processing 3 nucleotides downstream from the RegB-cleaved site in A-rich region downstream the SD sequence. The secondary cleavages within RegB-processed mRNAs from T4 gene *43* and gene *comCα* region have been observed previously (Hsu and Karam, 1990; Sanson and Uzan, 1993, 1995). Cleavages took place in AU-rich sequences six to eight nucleotides downstream of the cleaved GGAG sites but have never been detected downstream of cleavages in SD sequences, i.e., where an AUG initiation codon lies downstream of the processed site. Since RegB endoribonuclease destabilizes most of the T4 early mRNAs, it was suggested that primary processing by RegB provides entry sites for other RNases, leading to the degradation (Sanson and Uzan, 1993, 1995; Sanson et al., 2000). However, the endonuclease responsible for the cutting at these secondary sites has not been identified, while RNase E was proposed to be a good candidate.

In this study, we present evidence that the nucleolytic activity at the secondary sites of RegB-processed transcripts for gene *30.7* of TuIa and RB69 is attributable to the *E. coli* endoribonuclease E. Only the RegB-processed mRNAs are susceptible to the hydrolysis at the secondary sites. This confirms the hypothesis that RegB accelerates degradation of mRNAs by providing the substrates for recognition by other RNases, in this case—for the RNase E. The involvement of RNase E in the degradation of early phage mRNAs is not well

established. It was proposed that long polycistronic early mRNAs carrying multiple secondary structures exhibit some resistance to RNase E (Sanson et al., 2000).

E. coli RNase E is the 5′-end-dependent endoribonuclease (Mackie, 1998, 2000), which lacks strict sequence specificity and cleaves in a single-stranded RNA segments rich in A + U nucleotides (Lin-Chao et al., 1994; McDowall et al., 1994). Stem-loop structures can affect nearby cleavages by RNase E (Ehretsmann et al., 1992; Carpousis et al., 1994) but are not necessary (McDowall et al., 1995). It was proposed that stem-loops in the vicinity of RNase E cleavage sites might serve to stabilize local structure of RNA ensuring the single strandedness of the susceptible sites and their availability to the enzyme (Cormark and Mackie, 1992; Naureckiene and Uhlin, 1996). Attack by RNase E may be further modulated by translational efficiency (Arnold et al., 1998; Baker and Mackie, 2003; Braun et al., 1998; Joyce and Dreyfus, 1998). Untranslated mRNAs are more sensitive to the attack of this nuclease.

RNase E prefers RNA substrates that bear a monophosphate at an unpaired 5′-end, rather than otherwise identical RNAs bearing 5′-terminal triphosphate or a hydroxyl group (Feng et al., 2002; Jiang and Belasco, 2004; Mackie, 1998; Tock et al., 2000). The full-length primary transcripts for gene *30.7* of TuIa carrying 5′-triphosphorylated termini, strong SD element GGAG, and the canonical AUG initiation codon would be expected to direct translation initiation with high efficiency. Perhaps for these reasons, *E. coli* RNase E cannot cleave the primary transcripts of gene *30.7*. The cleavage within GGAG tetranucleotide by phage-coded RegB leads to reduced translational efficiency but produces the 5′-OH carrying RNAs (Uzan et al., 1988; Saïda et al., 2003). Nonetheless, numerous experiments have shown that *E. coli* RNase E is capable of slowly cleaving RNAs that bear 5′ triphosphate (Jiang et al., 2000; Mackie, 1998) or a 5′ hydroxyl group (Feng et al., 2002; Jiang and Belasco, 2004; Tock et al., 2000), or even capped RNA (Jiang et al., 2000). Thus, the RegB-cleaved RNAs can serve as the substrates for RNase E.

We supposed that the products of initial RegB cleavage within gene *30.7* mRNAs of TuIa and RB69 became susceptible to RNase E as a consequence of the reduced efficiency of translation. However, our results showed that untranslated full-length transcripts of this gene isolated from chloramphenicol-treated cells were resistant to RNase E (Fig. 7B). Furthermore, RNase E did not cleave the RegB-processed mRNA for gene *30.8* of RB69 carrying the same primary structure in the translation initiation region as the transcripts for gene *30.7*. Therefore, it is tempting to propose that RegB-induced reorganization of the higher order structure of the whole transcript modulates RNase E cleavage by providing or by limiting access of the enzyme to the susceptible sites.

According to the current knowledge, mRNA degradation in *E. coli* generally initiates with an endonucleolytic cleavage which triggers a cascade of other endonucleolytic cuts followed by degradation by exonucleases (Régnier and Arraiano, 2000; Steege, 2000). The initial cleavage either removes secondary structures present at the 5′-end of the message or prevents ribosome loading onto the mRNA, both of which are known to greatly destabilize the entire mRNA (Arnold et al., 1998; Baker and Mackie, 2003; Afonyushkin et al., 2005). The mRNAs reported to be stabilized by translation usually harbor the rate-limiting endonucleolytic cleavage sites, which are masked by ribosomes (Nilsson et al., 1987; Yarchuk et al., 1991; Iost and Dreyfus, 1995). RNase E frequently initiates the decay of many mRNAs (Cohen and McDowal, 1997; Jain and Belasco, 1995; Joyce and Dreyfus, 1998), but in some cases, the initial cleavage can be performed by RNase III (Portier et al., 1987; Bardwell et al., 1989).

Early viral messages that are synthesized immediately after infection should be protected from the host degradation enzymes. It is thought that early T4 messages contain fewer accessible cleavage or entry sites for the host RNases. Therefore, degradation of such mRNAs requires the RegB-mediated mechanism. The *regB* gene is widely conserved between T4 relatives indicating that RegB-mediated mechanism of degradation is common to T4-type phages. It was shown that the activity of the T4 RegB in vitro requires an enhancement by the *E. coli* ribosomal protein S1 (Ruckman et al., 1994; Lebars et al., 2001; Bisaglia et al., 2003), which plays an essential role in the initiation of translation. The association of RegB with the ribosomal proteins in vivo may help this RNase to overcome the stabilizing effect of the efficient translation initiation at the GGAG-carrying SD sequences. Then cleavage by RNase RegB might be considered as the first step, which promotes specific degradation of certain early mRNAs. Here, we present perhaps the first experimental evidence that the initial cleavage by RegB enables degradation of early phage mRNAs by the major *E. coli* ribonuclease E.

Materials and methods

Bacterial and bacteriophage strains

E. coli strain B^E (sup^0) was a gift from Dr. L. W. Black. *E. coli* strain CR63 (*supD*, ser) was kindly provided by Dr. K. N. Kreuzer. *E. coli* RNase E^+ and RNase E^- isogenic strains N3433 (genotype HfrH, lacZ43, lambda-, relA1, spoT1, thi-1) and N3431 (genotype N3433, rne3071(ts)) were kindly supplied by Dr. P. Régnier.

Phages T2, T6, M1, K3, Ox2, TuIa, and TuIb were obtained from Dr. U. Henning. Phages RB2, RB15, RB32, RB33, RB69, RB70, KC69, Pol, and LZ10 were kindly provided by Dr. K. Carlson. Bacteriophages T4D wild type was from Dr. W. B. Wood. Phage T4 $regB^-$ (*regBL52*) was a gift from Dr. M. Uzan. All phages were grown in *E. coli* B^E (sup^0), except for TuIb grown in *E. coli* CR63 (*supD*, ser).

PCR and sequencing procedures

Initially, the DNA fragments of the T4-related bacteriophages were amplified by PCR using the T4-specific primers. Phage plaques were used as a source of DNA templates for PCR amplification. The PCR protocol involved 30 cycles with

a program of denaturation at 92 °C for 1 min, primer annealing at 50 °C for 1 min, and extension at 72 °C for 1 min. In some cases, the annealing temperatures had to be adapted for some of these templates.

Sequencing was performed using a CycleReader DNA sequencing kit (Fermentas AB). The DNA template for the sequencing reactions was either in the form of a PCR fragments or in the form of a purified genomic phage DNA prepared for the direct sequencing as described by Kricker (1994). The oligonucleotide primers were 5′-end labeled by T4 polynucleotide kinase (Fermentas AB) with [γ-^{33}P]ATP or [γ-^{32}P]ATP (Amersham Biosciences). Phage-specific primers for PCR and sequencing procedures were used as follows.

T4-specific primers: Pr.1, 5′-GAGCACGTGCGGTTCTTCGAG (122–142 nt of gene *30.9*); Pr.2, 5′-GCCATTAAGAACCACATAGTTTC (complementary to 61–83 nt of gene *30.8*); Pr.3, 5′-GTTGGTAAACTTGACATGCAGC (292–313 nt of gene *30.8*); Pr.4, 5′-GTATCAATAGAACCTGCAATACC (complementary to 40–62 nt of gene *30.7*); Pr.5, 5′-GCTGGAATGGTGCTAATCG (242–260 nt of gene *30.7*); Pr.6, 5′-GCCTTTGAGAAAACCACTTCACAG (complementary to 39–62 nt of gene *30.6*); and Pr.7, 5′-CGGATAACCCAAATATACGACG (complementary to 151–172 nt of gene *30.3*).

RB69-specific primers: Pr.8, 5′-GGCTCCCTTTTGTGCGTTTAGG (16–37 nt of non-coding region between genes *30.9* and *30.8*); Pr.9, 5′-GGAGTCATGCACTGTTCGATTTCAC (complementary to 72–95 nt of gene *30.8*).

TuIa-specific primers: Pr.10, 5′-GGAGTCATGCACTGTTCGATTTCAC (complementary to 98–122 nt of gene *30.7*); Pr.11, 5′-GGCGTGGAGTAATATCCCCA (complementary to 42–61 nt of gene *30.6A*).

The nucleotide sequences have been submitted to the EMBL/GenBank database under the accession numbers: AJ318417 (bacteriophage T2), AJ318418 (T6), 318419 (M1), AJ315751 (K3), AJ315752 (Ox2), AJ315753 (TuIb), AJ315754 (RB2), AJ315755 (RB15), AJ315756 (RB32), AJ315757 (RB33), AJ315759 (RB70), AJ315758 (KC69), AJ315760 (Pol), AJ621579 (TuIa), AJ438770 (RB69) and AJ971414 (LZ10).

RNA preparation and primer extension analysis of phage mRNAs

Total RNA from phage-infected *E. coli* cells was phenol extracted and used for the primer extension analysis or RNA sequencing under conditions of primer excess, using avian myeloblastosis virus reverse transcriptase, as described (Uzan et al., 1988; Truncaite et al., 2003). A total of 5 synthetic oligonucleotides complementary to the coding sequences of genes of phages T4 (Pr.2, Pr.4, Pr.6), RB69 (Pr.9), and TuIa (Pr.10) were used to prime reverse transcriptase.

Assays of RegB endoribonuclease activity in phage-infected cells

To detect the RegB cleavage sites in the phage-induced transcripts, the *E. coli* B^{E} cells were grown at 30 °C to density 3 × 10^{8} cells/ml in LB medium and then were infected with phages T4, RB69, or TuIa (m.o.i. = 10) in the presence or in the absence of chloramphenicol. Chloramphenicol (150 μg/ml) was added 1 min prior to phage infection. The cells were collected 6 min after infection, lysed immediately, and total cellular RNA was purified. Cleavages were analyzed by primer extension sequencing.

RNase E cleavage assay

E. coli strains N3433 (rne^{+}) and N3431 (rne^{-}) were grown in LB medium supplemented with thymine (50 μg/ml) at 30 °C to A_{600} = 0.5. With or without shifting of the cultures to 43 °C for 30 min, cells were infected with TuIa or RB69 (m.o.i. = 10). The cells were withdrawn at 10 min after infection, lysed immediately, and total cellular RNA was purified. RNase E cleavage was determined by primer extension sequencing.

Acknowledgments

We thank Dr. Lindsay W. Black, Dr. Philippe Régnier, and Dr. Kenneth N. Kreuzer for *E. coli* strains, Dr. Karin Carlson, Dr. William B. Wood, and Dr. Marc Uzan for the phages. This work was supported in part by the Lithuanian State Science and Studies Foundation.

References

Afonyushkin, T., Večerek, B., Moll, I., Bläsi, U., Kaberdin, V.R., 2005. Both RNase E and RNase III control the stability of *sodB* mRNA upon translational inhibition by the small regulatory RNA RyhB. Nucleic Acids Res. 33, 1678–1689.

Arnold, T.E., Yu, J., Belasco, J.G., 1998. mRNA stabilization by the *ompA* 5′ untranslated region: two protective elements hinder distinct pathways for mRNA degradation. RNA 4, 319–330.

Baker, K.E., Mackie, G.A., 2003. Ectopic RNase E sites promote bypass of 5′-end-dependent mRNA decay in *Escherichia coli*. Mol. Microbiol. 47, 75–88.

Bardwell, J.C.A., Régnier, P., Chen, S.-M., Nakamura, Y., Grunberg-Manago, M., Court, D.L., 1989. Autoregulation of RNase III operon by mRNA processing. EMBO J. 8, 3401–3407.

Bernstein, H., Bernstein, C., 1989. Bacteriophage T4 genetic homologies with bacteria and eucaryotes. J. Bacteriol. 171, 2265–2270.

Bisaglia, M., Laalami, S., Uzan, M., Bontems, F., 2003. Activation of the RegB endoribonuclease by the S1 ribosomal protein is due to cooperation between the S1 four C-terminal modules, in a substrate-dependant manner. J. Biol. Chem. 278, 15261–15271.

Braun, F., Le Derout, J., Régnier, P., 1998. Ribosomes inhibit an RNase E cleavage which induces the decay of the *rpsO* mRNA of *Escherichia coli*. EMBO J. 17, 4790–4797.

Carpousis, A.J., Mudd, E.A., Krisch, H.M., 1989. Transcription and mRNA processing upstream of bacteriophage T4 gene 32. Mol. Gen. Genet. 219, 39–48.

Carpousis, A.J., Van Houve, G., Ehrestmann, C., Krisch, H.M., 1994. Copurification of *E. coli* RNase E and PNPase: evidence for a specific association between two enzymes important in RNA processing and degradation. Cell 76, 889–900.

Cohen, S.N., McDowal, K.J., 1997. RNase E: still a wonderfully mysterious enzyme. Mol. Microbiol. 23, 1099–1106.

Cormark, R.S., Mackie, G.A., 1992. Structural requirements for the processing of *Escherichia coli* 5S ribosomal RNA by RNase E in vitro. J. Mol. Biol. 228, 1078–1090.

Desplats, C., Krisch, H.M., 2003. The diversity and evolution of the T4-type bacteriophages. Res. Microbiol. 154, 259–267.

Deutscher, M.P., Li, Z., 2001. Exoribonucleases and their multiple roles in RNA metabolism. Prog. Nucleic Acid Res. Mol. Biol. 66, 67–105.

Ehretsmann, C.P., Carpousis, A.J., Krisch, H.M., 1992. Specificity of *Escherichia coli* endoribonuclease RNase E: in vivo and in vitro analysis of mutants in a bacteriophage T4 mRNA processing site. Genes Dev. 6, 149–159.

Feng, Y., Vickers, T.A., Cohen, S.N., 2002. The catalytic domain of RNase E shows inherent 3′ to 5′ directionality in cleavage site selection. Proc. Natl. Acad. Sci. U.S.A. 99, 14746–14751.

Gram, H., Liebig, H.D., Hack, A., Niggemann, E., Rüger, W., 1984. A physical map of bacteriophage T4 including the positions of strong promoters and terminators recognized in vitro. Mol. Gen. Genet. 194, 232–240.

Hambly, E., Tétart, F., Desplats, C., Wilson, W.H., Krisch, H.M., Mann, N.H., 2001. A conserved genetic module that encodes the major virion components in both the coliphage T4 and the marine cyanophage S-PM2. Proc. Natl. Acad. Sci. U.S.A. 98, 11411–11416.

Hsu, T., Karam, J.D., 1990. Transcriptional mapping of a DNA replication gene cluster in bacteriophage T4. Sites for initiation, termination, and mRNA processing. J. Biol. Chem. 265, 5303–5316.

Iost, I., Dreyfus, M., 1995. The stability of *Escherichia coli lacZ* mRNA depends upon the simultaneity of its synthesis and translation. EMBO J. 14, 3252–3261.

Jain, C., Belasco, J.G., 1995. RNase E autoregulates its synthesis by controlling the degradation rate of its own mRNA in *Escherichia coli*: unusual sensitivity of the *rne* transcript to RNase E activity. Genes Dev. 9, 84–96.

Jiang, X., Belasco, J.G., 2004. Catalytic activation of multimeric RNase E and RNase G by 5′-monophosphorylated RNA. Proc. Natl. Acad. Sci. U.S.A. 101, 9211–9216.

Jiang, X., Diwa, A., Belasco, J.G., 2000. Regions of RNase E important for 5′-end-dependent RNA cleavage and autoregulated synthesis. J. Bacteriol. 182, 2468–2475.

Joyce, S.A., Dreyfus, M., 1998. In the absence of translation, RNase E can bypass 5′ mRNA stabilizers in *Escherichia coli*. J. Mol. Biol. 282, 241–254.

Kai, T., Yonesaki, T., 2002. Multiple mechanisms for degradation of Bacteriophage T4 *soc* mRNA. Genetics 160, 5–12.

Kai, T., Selick, E.H., Yonesaki, T., 1996. Destabilization of bacteriophage T4 mRNAs by a mutation of gene *61.5*. Genetics 144, 7–14.

Kai, T., Ueno, H., Yonesaki, T., 1998. Involvement of other T4 genes in the blockade of protein synthesis and mRNA destabilization by a mutation of gene *61.5*. Virology 248, 148–155.

Kricker, M., 1994. Sequencing of genomic T4 DNA. In: Karam, J.D., Drake, J., Kreuzer, K., Mosig, G., Hall, D., Eiserling, F., Black, L., Spicer, E., Kutter, E., Carlson, K., Miller, E. (Eds.), Molecular Biology of Bacteriophage T4. American Society for Microbiology, Washington, DC, pp. 463–464.

Kushner, S.R., 2002. mRNA decay in *Escherichia coli* comes of age. J. Mol. Biol. 184, 4658–4665.

Lebars, I., Hu, R.-M., Lallemand, J.-Y., Uzan, M., Bontems, F., 2001. Role of the substrate conformation and of the S1 protein in the cleavage efficiency of the T4 endoribonuclease RegB. J. Biol. Chem. 276, 13264–13272.

Liebig, H.D., Rüger, W., 1989. Bacteriophage T4 early promoter regions. Consensus sequences of promoters and ribosome-binding sites. J. Mol. Biol. 208, 517–536.

Lin-Chao, S., Wong, T.-T., McDowall, K.J., Cohen, S.N., 1994. Effect of nucleotide sequence on the specificity of *rne*-dependent and RNase E-mediated cleavages of RNA I encoded by the pBR322 plasmid. J. Biol. Chem. 269, 10797–10803.

Loayza, D., Carpousis, A.J., Krisch, H.M., 1991. Gene 32 transcription and mRNA processing in T4-related bacteriophages. Mol. Microbiol. 5, 715–725.

Luke, K., Radek, A., Liu, X.P., Campbell, J., Uzan, M., Haselkorn, R., Kogan, Y., 2002. Microarray analysis of gene expression during bacteriophage T4 infection. Virology 299, 182–191.

Mackie, G.A., 1998. Ribonuclease E is a 5′-end-dependent endonuclease. Nature 395, 720–723.

Mackie, G.A., 2000. Stabilization of circular *rpsT* mRNA demonstrates the 5′-end dependence of RNase E action in vivo. J. Biol. Chem. 275, 25069–25072.

McDowall, K.J., Lin-Chao, S., Cohen, S.N., 1994. A + U content rather than a particular nucleotide order determines the specificity of RNase E cleavage. J. Biol. Chem. 269, 10790–10796.

McDowall, K.J., Kaberdin, V.R., Wu, S.-W., Cohen, S.N., Lin-Chao, S., 1995. Site-specific RNase E cleavage of oligonucleotides and inhibition by stem-loops. Nature 374, 287–290.

Miller, E.S., Heidelberg, J.F., Eisen, J.A., Nelson, W.C., Durkin, A.S., Ciecko, A., Feldblyum, T.V., White, O., Paulsen, I.T., Nierman, W.C., Lee, J., Szczypinski, B., Fraser, C.M., 2003a. Complete genome sequence of the broad-host-range vibriophage KVP40: comparative genomics of a T4-related bacteriophage. J. Bacteriol. 185, 5220–5233.

Miller, E.S., Kutter, E., Mosig, G., Arisaka, F., Kunisawa, T., Rüger, W., 2003b. Bacteriophage T4 genome. Microbiol. Mol. Biol. Rev. 67, 86–156.

Monod, C., Repoila, F., Kutateladze, M., Tétart, F., Krisch, H.M., 1997. The genome of the pseudo T-even bacteriophages, a diverse group that resembles T4. J. Mol. Biol. 267, 237–249.

Mudd, E.A., Prentki, P., Belin, D., Krisch, H.M., 1988. Processing of unstable bacteriophage T4 gene 32 mRNAs into a stable species requires *Escherichia coli* ribonuclease E. EMBO J. 7, 3601–3607.

Mudd, E.A., Carpousis, AJ., Krisch, H.M., 1990. *Escherichia coli* RNase E has a role in the decay of bacteriophage T4 mRNA. Genes Dev. 4, 873–881.

Naureckiene, S., Uhlin, B.E., 1996. In vitro analysis of messenger RNA processing by RNase E in the *pap* operon of *Escherichia coli*. Mol. Microbiol. 21, 55–68.

Nilsson, G., Belasco, J.G., Cohen, S.N., von Gabain, A., 1987. Effect of premature termination of translation on mRNA stability depends on the site of ribosome release. Proc. Natl. Acad. Sci. U.S.A. 84, 4890–4894.

Nivinskas, R., Zajančkauskaite, A., Raudonikiene, A., Viteniene, I., 1992. The nucleotide sequence between genes 31 and 30 of bacteriophage T4. DNA Sequence. J. DNA Sequencing Mapp. 2, 405–409.

Notredame, C., Higgins, D.G., Heringa, J., 2000. T-Coffee: a novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 302, 205–217.

Otsuka, Y., Yonesaki, T., 2005. A novel endoribonuclease, RNase LS, in *Escherichia coli*. Genetics 169, 13–20.

Otsuka, Y., Ueno, H., Yonesaki, T., 2003. *Escherichia coli* endoribonucleases involved in cleavage of bacteriophage T4 mRNAs. J. Bacteriol. 185, 983–990.

Piešiniene, L., Truncaite, L., Zajančkauskaite, A., Nivinskas, R., 2004. The sequences and activities of RegB endoribonucleases of T4-related bacteriophages. Nucleic Acids Res. 32, 5582–5595.

Portier, C., Dondon, L., Grunberg-Manago, M., Régnier, P., 1987. The first step in the functional inactivation of the *Escherichia coli* polynucleotide phophorylase messenger is a ribonuclease III processing at the 5′ end. EMBO J. 6, 2165–2170.

Régnier, P., Arraiano, C.M., 2000. Degradation of mRNA in bacteria: emergence of ubiquitous feature. Bioassays 22, 235–244.

Repoila, F., Tétart, F., Bouet, J.Y., Krisch, H.M., 1994. Genomic polymorphism in the T-even bacteriophages. EMBO J. 13, 4181–4192.

Ruckman, J., Parma, D., Tuerk, C., Hall, D.H., Gold, L., 1989. Identification of a T4 gene required for bacteriophage mRNA processing. New Biol. 1, 54–65.

Ruckman, J., Rinquist, S., Brody, E., Gold, L., 1994. The bacteriophage T4 *regB* ribonuclease. Stimulation of the purified enzyme by ribosomal protein S1. J. Biol. Chem. 269, 26655–26662.

Saïda, F., Uzan, M., Bontems, F., 2003. The phage T4 restriction endoribonuclease RegB: a cyclizing enzyme that requires two histidines to be fully active. Nucleic Acids Res. 31, 2751–2758.

Sanson, B., Uzan, M., 1993. Dual role of the sequence-specific bacteriophage T4 endoribonuclease RegB: mRNA inactivation and mRNA destabilization. J. Mol. Biol. 233, 429–446.

Sanson, B., Uzan, M., 1995. Post-transcriptional controls in bacteriophage T4: roles of the sequence-specific endoribonuclease RegB. FEMS Microbiol. Rev. 17, 141–150.

Sanson, B., Hu, R.M., Troitskaya, E., Mathy, N., Uzan, M., 2000. Endoribonuclease RegB from bacteriophage T4 is necessary for the degradation of early but not middle or late mRNAs. J. Mol. Biol. 297, 1063–1074.

Sommer, N., Salniene, V., Gineikiene, E., Nivinskas, R., Rüger, W., 2000. T4

early promoter strength probed in vivo with unribosylated and ADP-ribosylated *Escherichia coli* RNA polymerase: a mutation analysis. Microbiology 146, 2643–2653.

Steege, D.A., 2000. Emerging features of mRNA decay in bacteria. RNA 6, 1079–1090.

Tétart, F., Desplats, C., Kutateladze, M., Monod, C., Ackermann, H.W., Krisch, H.M., 2001. Phylogeny of the major head and tail genes of the wide-ranging T4-type bacteriophages. J. Bacteriol. 183, 358–366.

Tock, M.R., Walsh, A.P., Carroll, G., McDowall, K.J., 2000. The CafA protein required for the 5′-maturation of 16 S rRNA is a 5′-end-dependent ribonuclease that has context-dependent broad sequence specificity. J. Biol. Chem. 275, 8726–8732.

Truncaite, L., Zajančkauskaite, A., Nivinskas, R., 2002. Identification of two middle promoters upstream DNA ligase gene *30* of bacteriophage T4. J. Mol. Biol. 317, 179–190.

Truncaite, L., Piešiniene, L., Kolesinskiene, G., Zajančkauskaite, A., Driukas, A., Klausa, V., Nivinskas, R., 2003. Twelve new MotA-dependent middle promoters of bacteriophage T4: consensus sequence revised. J. Mol. Biol. 327, 335–346.

Tuerk, C., Gauss, P., Thermes, C., Groebe, D.R., Gayle, M., Guild, N., Stormo, G., d'Aubenton-Carafa, Y., Uhlenbeck, O.C., Tinoco Jr., I., Brody, E.N., Gold, L., 1988. CUUCGG hairpins: extraordinarily stable RNA secondary structures associated with various biochemical processes. Proc. Natl. Acad. Sci. U.S.A. 85, 1364–1368.

Ueno, H., Yonesaki, T., 2001. Recognition and specific degradation of bacteriophage T4 mRNAs. Genetics 158, 7–17.

Ueno, H., Yonesaki, T., 2004. Phage-induced change in the stability of mRNAs. Virology 329, 134–141.

Uzan, M., Favre, R., Brody, E., 1988. A nuclease that cuts specifically in the ribosome binding site of some T4 mRNAs. Proc. Natl. Acad. Sci. U.S.A. 85, 8895–8899.

Wilkens, K., Rüger, W., 1994. Transcription from early promoters. In: Karam, J.D., Drake, J., Kreuzer, K., Mosig, G., Hall, D., Eiserling, F., Black, L., Spicer, E., Kutter, E., Carlson, K., Miller, E. (Eds.), Molecular Biology of Bacteriophage T4. American Society for Microbiology, Washington, DC, pp. 132–141.

Wilkens, K., Rüger, W., 1996. Characterization of bacteriophage T4 early promoters in vivo with a new promoter probe vector. Plasmid 35, 108–120.

Yarchuk, O., Iost, I., Dreyfus, M., 1991. The relation between translation and mRNA degradation in the *lacZ* gene. Biochimie 73, 1533–1541.

Yeh, L.S., Hsu, T., Karam, J.D., 1998. Divergence of a DNA replication gene cluster in the T4-related bacteriophage RB69. J. Bacteriol. 180, 2005–2013.

Zuo, Y., Deutscher, M.P., 2001. Exoribonuclease superfamilies: structural analysis and phylogenetic distribution. Nucleic Acids Res. 29, 1017–1026.